APPENDIX 3.8.6.

PRELIMINARY GUIDELINES FOR THE ESTABLISHMENT OR THE REGAINING OF RECOGNITION FOR A FOOT AND MOUTH DISEASE FREE COUNTRY OR ZONE

Article 3.8.6.3.

Countries or zones applying for freedom from FMD where vaccination is not practised

1. Introduction

In addition to the general conditions, a Member Country applying for recognition of freedom from FMD where vaccination is not practised should show evidence of an effective surveillance programme in which either the FMD susceptible population undergoes regular clinical examination or a statistically significant sample of this population is examined to show that disease has not been present in the population during the past 12 months. In addition, a statistically significant proportion of the population should be subjected to serological surveillance to demonstrate absence of FMD virus (FMDV) infection during the preceding 12 months. This requires the support of a national or other reference laboratory able to undertake serology for FMDV antibody using tests described in the *Terrestrial Manual.*

2. Survey design

In general, the target population for random surveys for *disease* and *infection* will cover the susceptible species within the country or zone to be declared free from disease. Countries wishing to show freedom from FMD in which a pig-specific strain of virus had been prevalent should concentrate on sampling the national pig population. In countries in which an African buffalo population is present, this population should also be sampled if included in the proposed FMDV infection free zone.

The objective of the random sample design is to use the minimum level of surveillance consistent with demonstrating the absence of *disease / infection* at the required level of statistical confidence. The sample should be selected on a random basis during each of the consecutive sampling campaigns; the frequency of sampling is dependent on the epidemiological situation, but should be at least once during the year preceding the application. Every sampling unit should have an equal probability of being selected. The selection of individual sampling units should not affect the probability of selecting any other sampling unit. It should be emphasised that random selection of the sampling units is essential, or the required level of statistical confidence cannot be achieved.

In order to provide representative information on the infection status of the target population, the random sample survey ought to be completed within the shortest possible period of time.

The population may be divided into sections (strata) with similar epidemiological conditions within each stratum. Stratification implies that a suitable system of separating the target population into a series of sections or strata from which random samples can be drawn has been developed. A stratum should be a subpopulation of the total population that is raised using a similar production and husbandry system under similar ecological conditions within

geographical or administrative areas (provinces, states, etc.) with a similar likelihood of infection. Which stratification criteria will be most appropriate will depend on the conditions prevailing in the individual country.

During the process of stratification the following two conditions have to be met:

a) all sampling units (village, flock or herd depending on farming system) within a particular stratum can be accessed during the survey and have an equal chance of being selected;

b) an individual sampling unit is included in only one stratum.

The total number of strata required will depend on the country or zone concerned, and additional strata or an increased level of sampling may be applied to areas within a country or zone considered to be at a higher likelihood of FMDV infection. Care should be taken that the number of strata does not exceed the capacity of the field and laboratory service as the required number of random samples will have to be collected from each of the strata. The number of samples is determined, to a considerable extent, by the number of strata. Hence the number of strata should be kept to a minimum but also reflect major epidemiological differences. Further detail may be obtained from suitable epidemiological texts.

If a Member Country wishes to declare a specific zone within the country free from FMDV infection, this should be taken into consideration in the stratification process. The basis for the sampling process would then be the population within each zone.

The objective of the random sample survey is the detection of clinical or serological evidence of FMD within the population, if it is present at a predetermined prevalence. The probability of detecting evidence of FMD or FMDV infection in a given sample of animals depends on the prevalence of FMDV infection in the population and the size of the sample. Hence, the sample size and expected disease prevalence determine the level of confidence in the result of the survey. The lower the prevalence the larger the sample size has to be in order to achieve a given confidence in the outcome of the survey. The sampling strategy should give a 95% probability of detecting evidence of FMD or FMDV infection if it is present in 1% of the primary sampling units. In other words, if at least 1% of herds/flocks are infected with FMD virus, the sample size has to be large enough to give a 95% chance that at least one infected herd/flock will be detected through examination of the random sample of herds/flocks.

3. Clinical surveillance

Clinical surveillance aims at the detection of clinical signs of FMD by close inspection of the mouth, feet and udder of a randomly selected sample. It is essential that all animals within the selected primary sampling unit are examined for signs of FMD. Any herd/flock where suspicious animals are detected should be classified as infected until contrary evidence is produced.

4. Serological surveillance

Serological surveillance aims at the detection of antibodies against FMDV. A positive reaction to an FMDV antibody detection test can have four possible causes:

a) natural infection with FMDV;

b) vaccination;

c) maternal antibodies from an immune dam (maternal antibodies in cattle are usually found only up to 6 months of age, however, in some individuals and in buffalo calves, maternal antibody can be detected for longer);

d) non-specific reactions, for example to some other unrelated antigen (heterophile reactions).

Thus antibodies detected in animals (other than African buffalo) over 6 months of age and born after a country or region has ceased vaccination should be in response to natural infection and be indicative of circulating virus. This group of animals will be considered eligible as secondary sample units for the purpose of serological surveillance. It may be possible to use serum collected for other survey purposes, but the objective of a statistically valid random survey for the specific presence of FMDV should not be compromised.

If vaccination cannot be excluded as the cause of positive serological reactions, additional testing for the presence of antibodies to the nonstructural proteins (NSPs) of FMDV could indicate the previous presence of live FMDV.

It is unusual to find only one or two sero-positive animals in an infected herd/flock. For this reason and for practical as well as economic reasons, it is considered acceptable to include only a random sample of animals from each primary sampling unit in the serological surveillance. The sample size has to be sufficient to achieve a 95% probability of detecting sero-positive animals. If a herd is infected a significant time after the cessation of vaccination, it would be expected that the serological prevalence will exceed 20%.

FMDV persists in the pharyngeal region of recovered ruminants for up to 3 years in cattle and 9 months in sheep, and therefore oesophageal–pharyngeal (OP) fluid sampling is an additional valuable tool in surveillance for FMDV. OP samples should be collected from herds and flocks selected by positive serology. The collection of OP samples will depend on the availability of collection equipment (e.g. probang), facilities for storing the OP material until testing, and access to a laboratory able to work with live FMDV. Sheep can also be sampled by collecting OP fluid, and a similar sampling strategy can be applied, bearing in mind that the carrier state is shorter in this species.

Staff collecting OP samples should be given specific training on the techniques for the collection, transport and storage of OP fluid. It is essential that the OP fluid is placed in a neutral buffer and immediately frozen in or over liquid nitrogen or solid CO_2 after collection, and kept in this state until thawed in the diagnostic laboratory and placed on susceptible tissue culture (see the *Terrestrial Manual*).

It is preferable to stratify the sampling frame to reflect the possibility of FMD having been present up to 3 years previously. OP samples should be collected from each group of yearlings, 2-year-old and 3-year-old cattle/sheep in the selected herds and flocks.

The results of the random sample survey will provide evidence both to the national authorities and to the OIE that no FMDV infection is present in the country or zone. It is therefore essential that the random sample survey can be audited through clear documentation and the presence of complete records.

Article 3.8.6.6.

The use and interpretation of serological tests (see Fig 1)

The recommended serological tests for FMD surveillance are described in the *Terrestrial Manual.* In unvaccinated populations, the screening can be carried out using the liquid-phase blocking ELISA (LPBE) or the solid phase competition ELISA (SPCE). The sensitivity of the LPBE approaches 100% but it can have a specificity in cattle as low as 95%, and will therefore give up to 5% false positive results at a titre greater than 40. Because the objective of the survey is to discover evidence of infection if the latter is present, it is acceptable for the purposes of the survey to raise the cut-off value for negative/positive sera. The rationale for raising or lowering the cut-off titre should be given in reports of tests for which this has been used. Raising of the cut-off value may still result in false positive results, and therefore positive sera should be re-tested by the virus neutralisation test (VNT), in which a titre of 45 or greater is classified as positive. Any animals whose sera are positive by the VNT should be re-sampled to confirm this status, and if still positive they should be tested for evidence of infection. The remaining animals in the herd/flock should also be tested for the presence of antibodies to FMDV and, if found positive, sampled by collection of OP material using a probang cup. The SPCE has been shown to have a higher specificity, but similar sensitivity to the LPBE, and should be used in preference to the LPBE where possible.

For serological surveillance in countries or zones in which vaccine is, or has been used, the LPBE or SPCE can still be the test of choice in those FMD susceptible species not included in the vaccination programme. Animals that have been vaccinated will have antibodies to the structural proteins of FMD virus, and some may have antibodies to the NSPs, depending on the number of times they have been vaccinated, and the amount of the NSPs present in the vaccine used. However, animals that have recovered from infection with FMD virus will have high levels of antibody to the NSPs. There are eight NSPs associated with the replication of FMD virus, namely L, 2A, 2B, 2C, 3A, 3B, 3C and 3D, and antibodies can be found to all of these in most recovered animals. Some do not persist for more than a few months, and some animals may fail to produce detectable levels to all of them. ELISA tests have been developed to detect 2C, 3B or 3ABC antibodies, the former being detectable for up to one year after infection, and the latter for up to 2 years. A western blot technique (EITB) has also been used to detect the NSP antibodies to 2C, 3ABC, 3A, 3B and 3D; it is particularly specific and sensitive in identifying animals previously infected. All these tests have been validated in cattle.

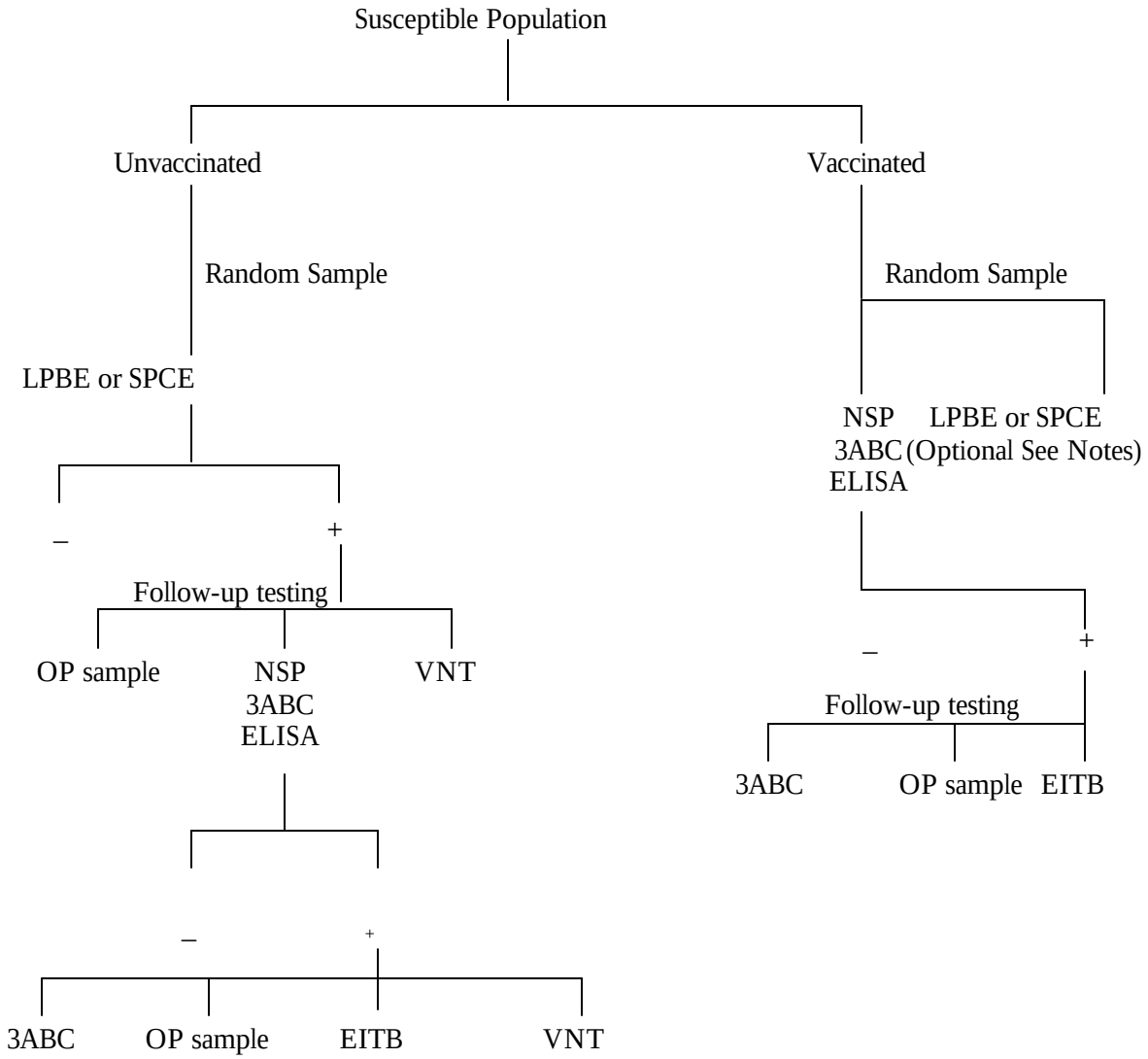
A class of animal exists, however, that has been infected with FMD virus and could remain carrying the virus without developing detectable antibodies to the NSPs. These are animals which have received highly potent vaccine and then had contact with the virus during an *outbreak*, but, because of their level of immunity, suppress viral replication and show no evidence of disease. Because the virus does not replicate significantly in these animals, there is little expression of the NSPs and therefore development of detectable levels of antibodies may not occur. However, on a herd basis there are always less protected animals following vaccination, and if these animals are challenged with the virus, they will produce antibodies to the NSPs, and can develop clinical disease. It is therefore important that NSP antibody tests be interpreted by assessing the level of these antibodies in the sera of a representative sample from the whole herd.

There is the option to use the NSP antibody test together with the LPBE or SPCE, particularly in areas where vaccination has been used and virus activity is suspected. LPBE titres or SPCE inhibition

higher than would be expected from vaccination alone, may suggest FMDV infection and this can be confirmed by testing for the presence of antibodies to the NSPs, and by taking OP samples.

The diagnostic sensitivity of tests used influences the numbers of animals that need to be sampled in a survey to provide evidence of absence of infection. The diagnostic specificity of the test influences the proportion and number of positive results to be expected in the absence or presence of infection, and therefore the selection and use of confirmatory tests. Results of surveys which indicate a significantly higher proportion of positive test results in comparison with that expected from the estimate of the false positive rate derived from the diagnostic specificity (i.e. 100 minus diagnostic specificity) may be interpreted as evidence of infection in the population and therefore a confirmatory test of high specificity, and where appropriate other investigations, should be conducted.

Fig. 1 ***Schematic representation of laboratory tests for determining evidence of FMDV infection through, or following serological surveys***



The above diagram indicates the tests which are recommended for use in the investigation of sampling units in which a positive test result has been obtained.

Key:

ELISA	enzyme-linked immunosorbant assay
LPBE	liquid-phase blocking ELISA
SPCE	solid-phase competition ELISA
VNT	virus neutralisation test
NSP	nonstructural protein(s) of FMDV
3ABC	NSP antibody test
EITB	western blot for NSP antibodies of FMDV
OP	oesophageal–pharyngeal sample

Figure 1 provides a flowchart of the test protocol that could be used to test the samples collected in the random survey. If the population being tested has not been previously vaccinated against FMD, the serum samples can be tested using the LPBE or SPCE. Sera positive on the test used should be retested using the VNT, which is the "gold standard" test for FMDV antibodies. In addition, or in place of the VNT if the laboratory is not able to manipulate live FMDV, the positive sera may be retested using a NSP antibody test, such as the 3B, 3ABC or EITB. If the positive sera are from a ruminant species, OP samples may also be collected and tested for the presence of live FMDV. A positive VNT or NSP test would indicate that live virus had been circulating, and would require further investigation of the herd or flock to show whether it was still present; a positive OP sample would provide definitive evidence. Further investigation should include serum testing of the whole herd or flock from which the positive samples were obtained, in addition to taking further OP samples to show whether live virus is still present.

NSP tests should be used for testing sera from vaccinated herds or flocks, as such sera will be positive by VNT. LPBE and SPCE can be used in addition, as described above. 3ABC or 3B positive samples may be repeat tested using the EITB for confirmation. All animals from herds and flocks from which positive samples are obtained should be re-tested for antibodies to NSP's, and OP samples collected for detection of live FMDV.

Data on the sensitivity and specificity of the NSP tests currently available is not fully documented, in particular for species other than cattle, or for vaccinated animals carrying live FMDV. However, this is under investigation in a number of laboratories worldwide. Member Countries submitting data to the OIE Scientific Commission for Animal Diseases which have been derived using commercial or other NSP tests should provide information on the characteristics of the test being used, and adjust the number of samples collected to accommodate the test parameters. In addition, the testing of OP samples for the presence of FMDV may be less than 50% sensitive, even using very sensitive tissue culture such as primary bovine thyroid cells or lamb kidney cells. If the initial attempt at virus isolation is negative, either repeat OP samples should be collected from serum-antibody positive animals after a 2-week interval, or further tests such as PCR carried out on the samples.